

Figure

Changed

Primer R

↑ pase¢↑

CACCGCTTGCCCCCAGAATGGATGCGCATGTCTG
CACCGCTTGCCCCCAGAATGGAGGGGGGTGTCTG
TATTACTGGGCGAGGTGTCCT.GTGGCGAACGGGGGTCTTACCTCCCACAGACATAA¥GACCCGCTCCACAGGA.

Target Sequence

Polymorphic

nucleotide

PCR amplify

Fok I/Fsp I

CTTGCCCCCAGAATGGATGCGCATGTCTG TATTACTGGGCGAGGTGTCCT.. GAACGGGGGTCTTACCTCCCACAGACATAATGACCCGCTCCACAGGA.

Digest with Fok I and Fsp I

8 mer GCATGTCT 67ATTACTGGGCGAGGTGTCCT... CCCACAGACATA ATGACCCGCTCCACAGGA.. CTTGCCCCCAGAATGGATGC GAACGGGGGTCTTACCTCCT

Figure 2

Cut with Fok I

Fsp I

↑ nnnnnnTGCGCAnnnnn nnnnnACGCG∓nnnnn Cut with Fsp I

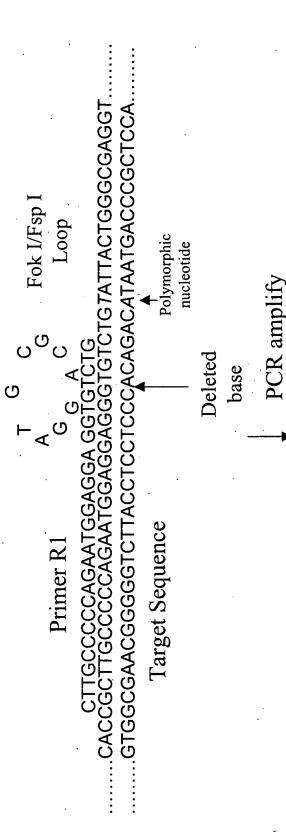
nnnnnnTGC GCAnnnnnn nnnnnnACG CGTnnnnn

Fok I GGATG CCGAC

TGCGCA FSp.I. ACGCGT Combined Fok I and Fsp I site

GGATGCGCA Fok I/Fsp I CCGACGCGT

Cosser espectable Figure 4



CTTGCCCCCAGAATGGAGGAGGATGCGCAGGT&TCTG7ATTACTGGGCGAGGT. GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA. Digest with Fok I and Fsp I

Fok I/Fsp I

CTTGCCCCCAGAATGGAGGAGGATGC GCAGGTGT CTG7ATTACTGGGCGAGGT. CGTCCACAGACA TAATGACCCGCTCCA... GAACGGGGTCTTACCTCCTCCTACG

posmor emozono Figure 5



CTGCAAAAGGATTTAT AAACTTC GACGITITCCIAAA TAITIGAAG 16 mer TGGCTGGAGTTGCGCTAGCAAGACGTGCAG ACCGACCTCAACGCGATCGTTCTGCACGTC

Digest with Bsg I and Pvull

Figure 6

ĝ က် Pvull/Fok I CGCCTATGGCTGGAGTTGCGCTAGCAAGGACCAAAAGGATTTATAAACTTC GCGGATACCGACCTCAACGCGATCGTTCTGGTTTTCCTAAATATTTGAAG тевствеваттесеставсавасса абеватта PCR amplify ດ໌ ŝ

Digest with Pvu II and Fok

CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAGC†GGATGAAGGA†TTA*T*AAACTTC GCGGATACCGACCTCAACGCGATCGTTCTGGTGTCGACCTACTTCCTAAATATTTGAAG

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CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAG CTGGATGAAGGATTTA TAAACTTC GCGGATACCGACCTCAACGCGATCGTTCTGGTGTC GACCTACTTCCTAAATATTT GAAG 16 mer

Comment and Figure 7

Fok I/Fsp I

GAACGGGGGTCTTACCTCCTCCT<mark>ACGCGTCCAC</mark>AGACATAATGACCCGCTCCA.... CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTG7ATTACTGGGCGAGGT.

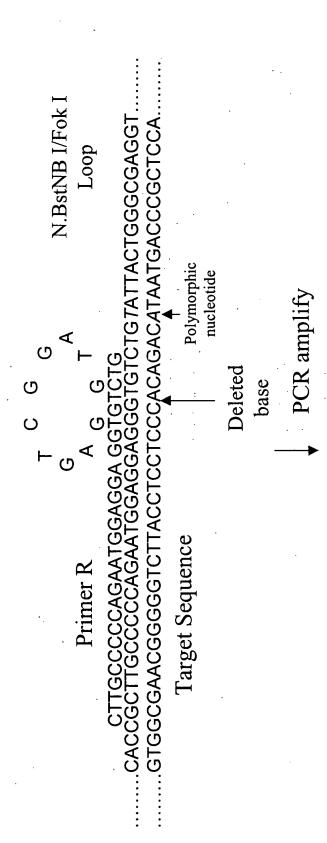
Remove nucleotides and digest with Fok I

CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGT GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA

Fill in with mass
Modified nucleotide

CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTmod GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA bcg 1 nnnnnnnnnnnnnCGAnnnnnTGCnnnnnnnnnnnnn nnnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnnnn Cleave with Bcg I

Domination of the British of the Bri

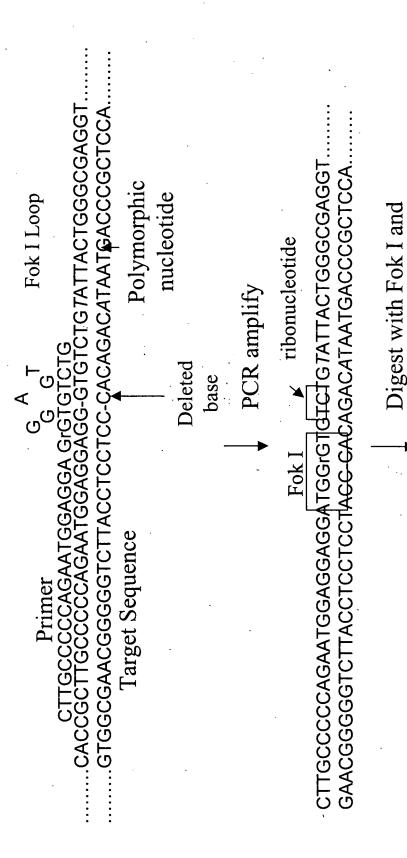


GAACGGGGGTCTTACCTCCTCTdAGCCTACCCACAGACATAATGACCCGCTCCA.. CTTGCCCCCAGAATGGAGGAGA&TCGGATGGGTGTCTG*T*ATTACTGGGCGAGGT Digest with Fok I and N.BstNB

N.BstNB I/Fok I

CTTGCCCCCAGAATGGAGGAGAGTCGGAT GGGTGTCTG $\it T$ ATTACTGGGCGAGGT. GACCCGCTCCA.. GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAAT

Comment and the Figure 10 .



T mer CTTGCCCCCAGAATGGAGGATGGrG TGTCTG7 ATTACTGGGCGAGGT GACCCGCTCCA. GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAAT

cleave with base

Figure 11. Methods for haplotyping based on physical allele separation

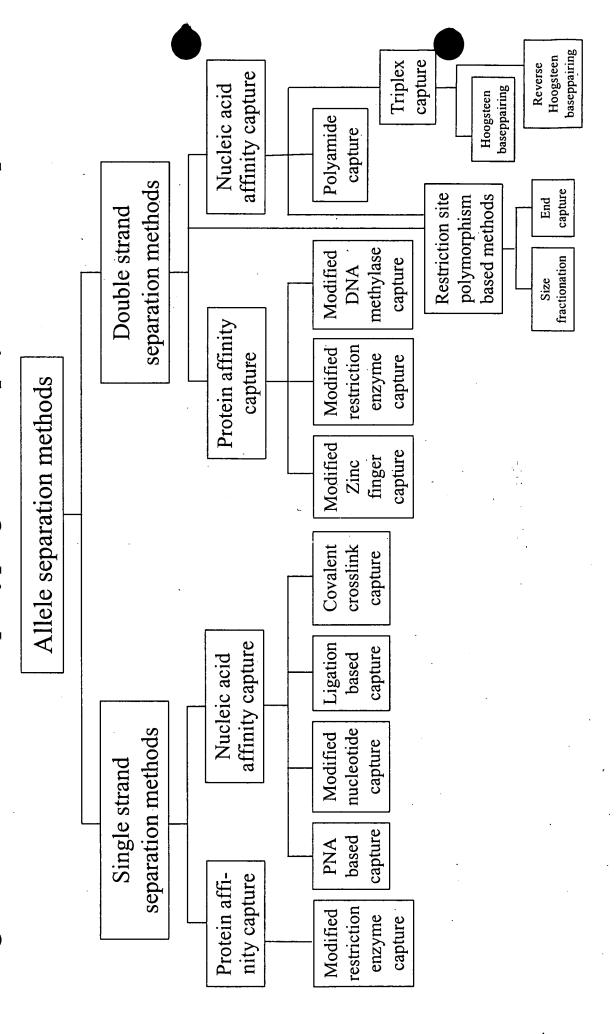


Figure 12. Methods for haplotyping based on allele specific amplification

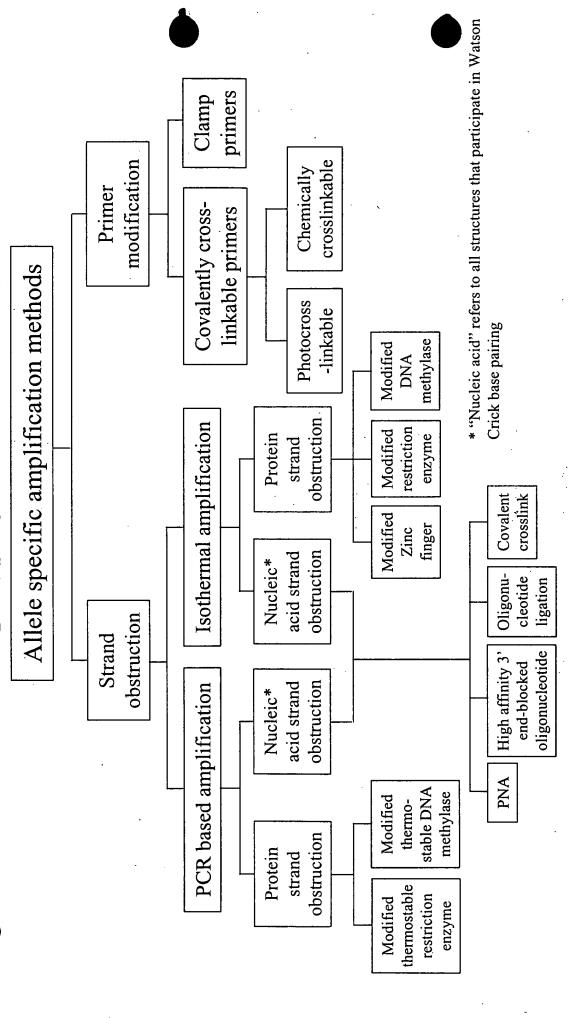
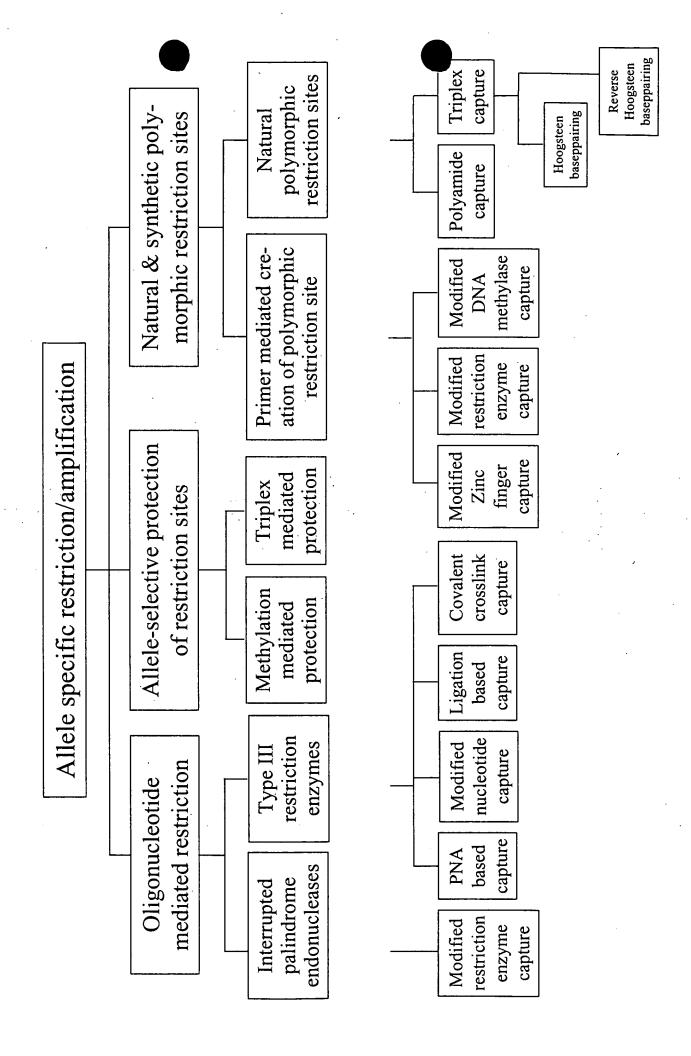


Figure 13. Methods for hablotybing based on allele specific restriction



D9697028 .1.C2500

Figure 14: Hairpin PCR Primers

	ATCTEGANNINNINNINTCCGGAT	MIMEN	ALLELE 2 T PRIMER	AGGCCTA	ATCTGGANNINNINNINNINTCC			TAGACCTNNNNNNNNNAGGTCTA	ATCTGGANNINNINNINNINTCCAGAT	ALLELE 1 F PRIMER	AGGTCTA	ATCTGGANNININININININITCC
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Figure 15: Hairpin PCR Primers

ATCCGGANNNNNNNNNNNN TCC	
ALLELE 1 C PRIMER	PCR Amplify
ATCCGGANNNNNNNNNNNTCCAGAT TAGGCCTNNNNNNNNNNAGG <i>T</i> CTA	
ATCCGGANNNNNNNNNNNNTCC	
ALLELE 2 C PRIMER	PCR Amplify
ATCCGGANNINNINNINNINTCCGGAT TAGGCCTNINNINNINNINAGGCCTA	

Figure 16: Hairpin PCR Primers

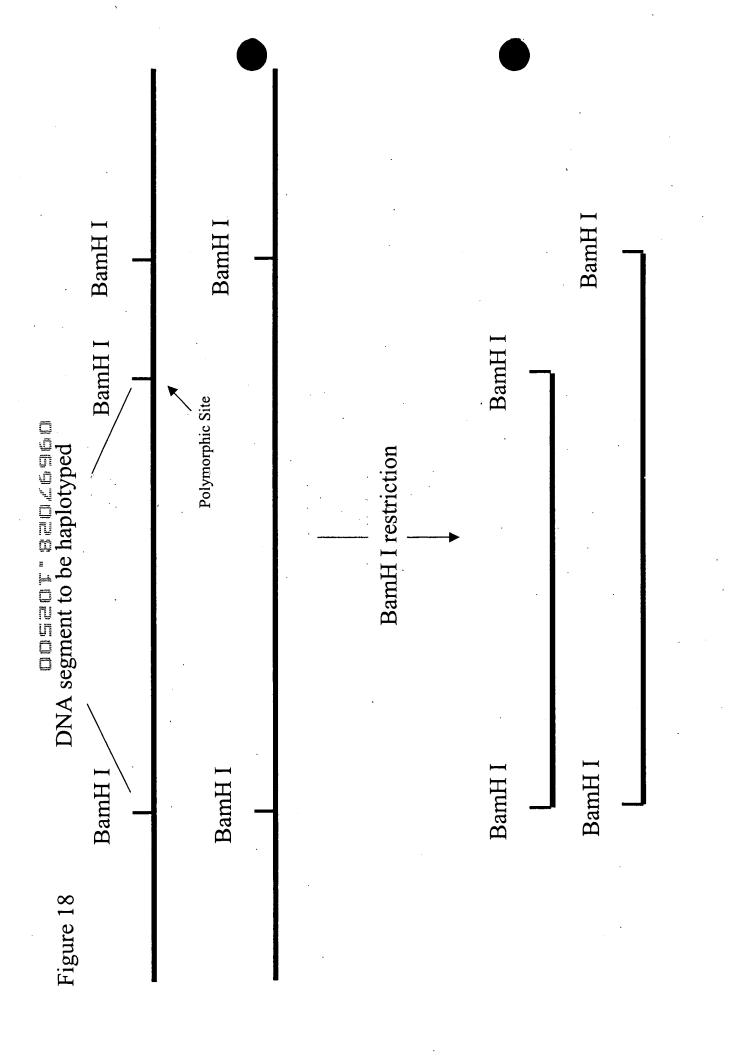
of allele 1
of 6
n PCR
g fror
Minus strand resulting from PCR of
strand
Minus

"C 3		þ	of ele 2 d
airpin loop forms inhibiting ybridization of PCR primer nd amplification of allele 1 Minus Stranc	PCR of allele 2	Minus Stran	Hairpin loop doesn't form due to mismatch allowing hybridization of PCR primer and amplification of allele 2 Minus Strand
——————————————————————————————————————	from]		<u> </u>
	ulting		
^J N TCCAGAT AGGTCTA	finus strand res	INNNAGGCCTA	N N N A TCC GAT A AGGCCTA N N N N
	2	NNNN	N N N N N N N N N N N N N N N N N N N
		TAGACCTNNNN	ALLELE 2 T PRIMER
	ALLELE 1 N NN N TCCAGAT and amplification of allele 1 N N NN N AGGTCTA Minus Strand	N TCCAGAT N AGGTCTA N N NN N AGGTCTA Minus strand resulting from PCR of allele 2	N N N N AGGTCTA N N N N AGGTCTA N N N N N N N N N N N N N N N N N N N

Figure 17: Hairpin PCR Primers

Minus strand resulting from PCR of allele 1
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NNNAT	TAGACCTALELE 2 C PRIMER
Minus strand resulting from PCR of allele 2	
N TCC GAT N N NN N AGGTCTA N N NN N N NN N N N NN N N N N N N	ALLELE 1 C PRIMER
TAGGCCTNNNNNNNNNAGGTCTA	TAGGCCT



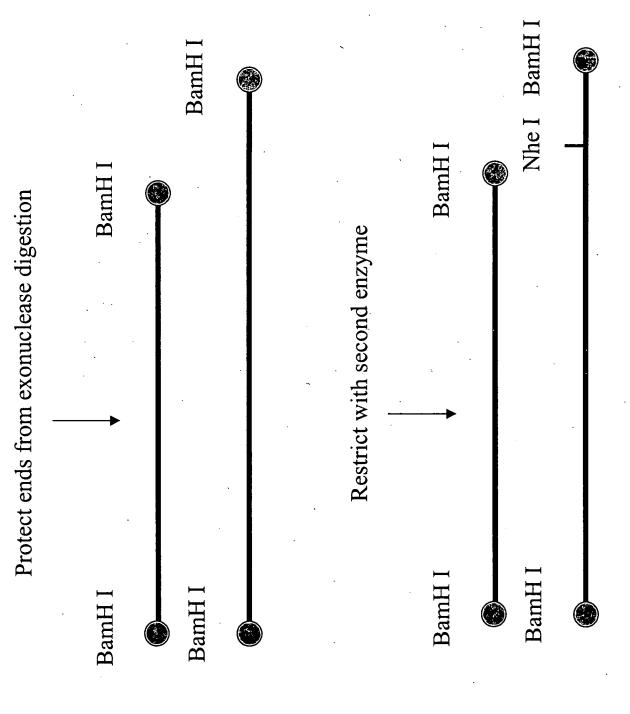


Figure 20

Digest with exonuclease

Add single strand nuclease to remove/degrade remaining single strand

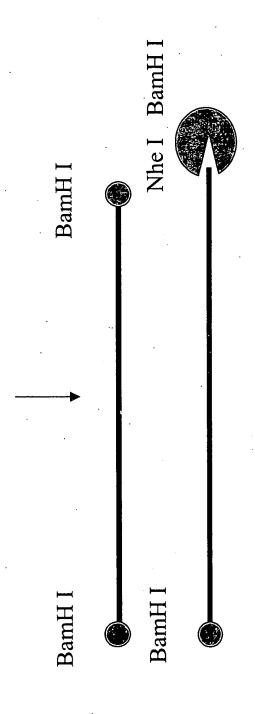
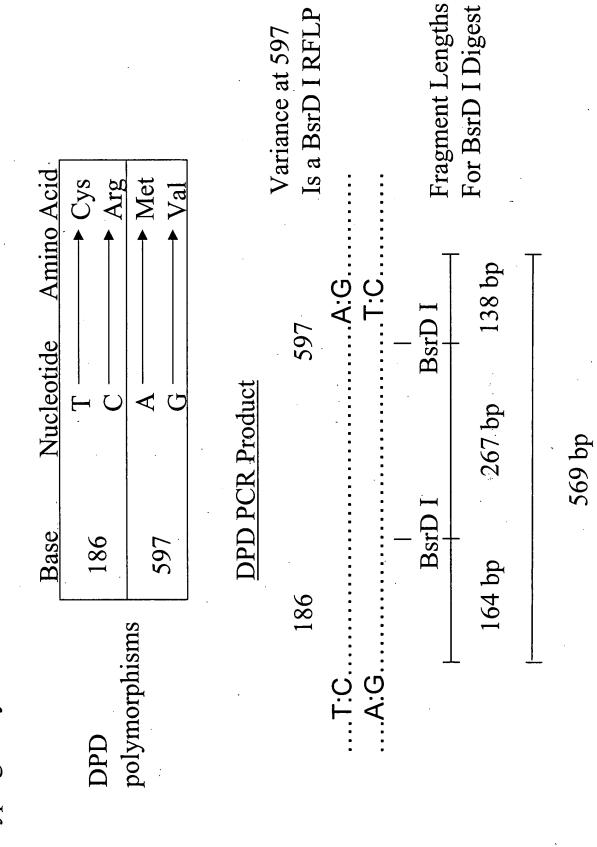


Figure 21. Dihydropyrimidine dehydrogenase (DPD) polymorphisms used in haplotyping assay.



Α.		í
ļ	DPDASCF	³ acacagactcatgcaactctg
DPD Primers	DPDASTF	5' <u>acqcaq</u> actcatgcaactctg
	DPDNSF	5' actcatgcaactct

DPD Sequence

5' actcatgcaactctg[T or C]gttccacttcggccaagaa, tgagtacgttgagac[A or G]caaggtgaagccggttctt

Z

Figure 23. PCR Amplification USing DPDNSF-Printer

5 actcatgcaactctg 3,	5'actcatgcaactctg 3' 	5'actcatgcaactctgTgttccac3, 	5, actcatgcaactctgCgttccac
DPDNSF primer	DPDNSF primer	T allele	C allele
Template: T allele	Template: C allele	PCR Product	PCR Product

Figure 24. PCR Amplification Using DPDASTF Primer.

		3, 5,	3,
5' <u>acgcagactcatgcaactctg</u> 	5' <u>acgcag</u> actcatgcaactctg 	5, acacagactcatgcaactctgTgttccac	5, acgcagactcatgcaactctgCgttccac TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
DPDASTF primer Template T allele	DPDASTF primer Template C allele	T allele PCR Product	C allele PCR Product

Figure 25. PCR Amplification Using DPDASCF Primer

$\frac{3^{\circ}}{acacagactcatgcaactctg}$ $\frac{3^{\circ}}{ f f f f }$ $\frac{3^{\circ}}{ f f f }$ $\frac{3^{\circ}}{ f f }$	$\begin{array}{ll} 5' \underbrace{acacagactcatgcaactctg} & 3' \\ \\ 3 ; \\ \vdots & \vdots \\ 3 ; \\ \end{array}$	5, acacagactcatgcaactctgTgttccac	5, acacagactcatgcaactctgCgttccac
DPDASCF primer	DPDASCF primer	T allele	C allele
Template T allele	Template C allele	PCR Product	PCR Product

COSTONE LOCUIO

Figure 26. Hairpin Structures for PCR Products Generated Using DPDNSF Primer

Hairpin Structure T Allele Reverse Strand

Hairpin Structure C Allele Reverse Strand

Figure 27. Hairpin Structures for PCR Products Generated Using DPDASCF Primer

Hairpin Structure T Allele Reverse Strand

Hairpin Structure C Allele Reverse Strand

$$Tm = 42$$
°C

Figure 28. Hairpin Structures for PCR Products Generated Using DPDASTF Primer

Hairpin Structure T Allele Reverse Strand

$$Tm = 42$$
°C

Hairpin Structure C Allele Reverse Strand

ALLELE C

DPDNSF primer 5' actcatgcaactctg 3' Tm = 41° C

$$Tm = 40^{\circ}C$$

ALLELE T

DPDNSF primer
$$5$$
' actcatgcaactctg 3 ' $Tm = 41$ °C

ALLELE C

DPDASCF primer $Tm = 60^{\circ}C$ $\frac{3'}{acacagactcatgcaactctg}$

atgagt
$$\frac{3}{|\ |\ |\ |}$$
 $Tm=42$ cgttgagacGcaaggtg......

Primer
Hybridization
and Amplification

5, acacagactcatgcaactctg 3, tototctgatctgagtacgttgagacGcaaggtg...

ALLELE T

DPDASCF primer $Tm = 60^{\circ}C$ acacagactcatgcaactctg

Hairpin inhibits
Primer Hybridization
and Amplification

5' acacagactcatgcaactctg 3'

ALLELE C

DPDASTF primer $Tm = 65^{\circ}C$

5' acgcagactcatgcaactctg

Hairpin inhibits primer hybridization and Amplification

5' acgcagactcatgcaactctg

ALLELE T

DPDASTF primer Tm = 65°C acgcagactcatgcaactctg

 $\text{atgagtctgcqt} = \frac{3}{1-1-1}$ Tm = 42 cgttgagacAcaaggtg.....

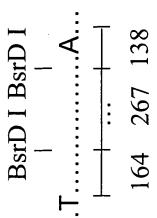
Primer hybridizes and amplification ensues

Figure 32. Allele Specific Amplification of a Heterozygous Sample with Haplotype T¹⁸⁶, A⁵⁹⁷ and C¹⁸⁶, G⁵⁹⁷

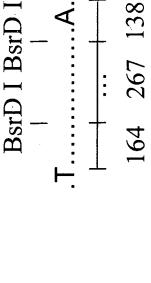
DPDNSF PRIMER

DPDASTF PRIMER

DPDASCF PRIMER



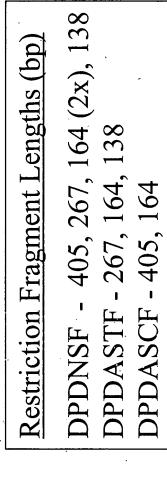
BsrD |











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Figure 33. BsrD I Digest of Allele Specific PCR Products.

DPDASCF DPDASTF PPDASTF PPDASCF

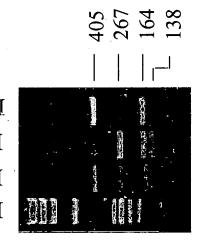


Figure 34

Fok I/Fsp] Loop ApoE21250-LR G CCCGGCTGGGCGCGGCACATG AGGACGTG

GCAGGCCCGGCTGGGCGCGCGGACATGGAGGACGTGTGCGGCCGCCTGGTGCAGTACCGC

Target Sequence

GGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCGCCT CCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGCGGA

Target Sequence

GGTGGACGCGTTCGACGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG GGCTACTGGACGTC CCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC

ApoE21250-LR

COSTONE LIONSID

Figure 35

T Allele Amplicon

CCCGGCTGGGCGCGGACATGCGCAAGGACGTGTGCGGCCGCCTGGTGCAGTAC GGGCCGACCCGCGCCTGTACCCTACGCGTTCCTGCACGCCCGGCGGACCACGTCATG CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCG GCGCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGC

GGAGGTGGACGCGTTCGACGCATTCGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC

C Allele Amplicon

CCCGGCTGGGCGCGGACATGGGATGCGCAAGGACGTGCGCGGCCGCCTGGTGCAGTAC GGGCCGACCCGCGCCTGTACCCTACGCGT#CCTGCACGCGCCGGGCGGACCACGTCATG CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCG GCGCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCCACGCGGAGC

GGAGGTGGACGCGTTCGACGCATTCGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC